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During this project period we made significant strides in understanding the role of the brain nucleus locus coeruleus (LC) in adaptive behavioral responsiveness. Progress included (i) substantial improvement of techniques to record LC neurons, and to make local microinjections into the LC, in behaving monkeys, (ii) analyses of LC responsiveness to target cues during performance of a visual discrimination/vigilance task, (iii) analyses of changes in tonic LC activity with changes in attentiveness during task performance, (iv) characterization of LC activity and behavioral performance during acquisition (learning) a new cue-contingency during reversal training, (v) analysis of effects of local pharmacological activation of LC neurons on vigilance performance and attentiveness, (vi) development of a neural network model of LC function, and (vi) improvement in techniques to retrogradely label afferents to monkey LC. Together, these studies have led to a new hypothesis of LC function, indicating that the LC serves to regulate neuronal and behavioral responsivity across a broad spectrum so as to promote either focused, task-defined performance or states of more labile, scanning attention which are optimal for changing, unpredictable environments.

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Dr Genevieve M. Haddad	(202) 767-5023	NL

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During our work on this project supported by AFOSR, we have extended our study of cellular mechanisms underlying vigilance and selective behavioral responsivity in primates. We have improved techniques and throughput for recording discharge of noradrenergic locus coeruleus (LC) neurons in brain during performance of a vigilance task that resembles those used in human psychophysical studies. Some results have confirmed our previous ideas about this system, but other results were unexpected and have indicated important new avenues of work to understand the role of the LC system in attention and vigilance. *These new results have led us to a more specific hypothesis of LC function in attention and vigilance, that is, that the LC regulates the lability of attention.* In the present application we propose to test this hypothesis and to extend our studies of LC function in the primate using anatomic experiments to identify afferent projections to the primate LC nucleus. The following studies are proposed: (1) We will record monkey LC neural activity during an attentional disengagement task, designed to allow manipulation and measurement of attentional detachment. *This will allow analysis of LC's involvement in attentional lability.* (2) Local microinjections of selective pharmacologic agents into the LC will be used to transiently and specifically inactivate or activate LC neurons during either the attentional detachment task, or a successive oddball discrimination task. The effects of these selective manipulations on short-term fluctuations in attention (measured via foveation of a fix spot required to initiate each trials in each task) will be determined. Effects will also be discerned on behavioral responses that reflect attentional lability and sustained attention in the two tasks. These experiments will *test LC's causal role in focused and labile attention, and determine whether different levels of LC activity are sufficient or necessary for such attentional processes.* (3) Environmental or cognitive stressors will be administered to determine their effect on LC activity in the waking primate, and to *test the role of LC in mediating the effects of stress on attentional performance.* Pupillary diameter and heart rate will be continuously monitored throughout recordings to ascertain stressful properties of the manipulation employed. (4) A retrograde tracer will be microinjected into the LC nucleus to identify afferent neurons. Sections containing labeled neurons will also be stained using an antibody to identify neurons that contain serotonin, norepinephrine, or adrenaline. These studies are an extension of our work over the past 7 years identifying afferents to the rodent LC, and will be *the first examination of inputs to the LC nucleus in the primate*..

The proposed studies will examine in detail both the temporal association (via LC recordings) and functional dependency (via LC activation and inactivation) between the brain noradrenergic LC system and attentional performance during normative as well as during stressful conditions. They will also identify neurons afferent to the LC, a necessary step in understanding the circuits and mechanisms involved in processing the specific stimulus attributes (novelty, expectancy, or saliency) that control LC activity and thereby regulate adaptive behavioral responsiveness.

Status of Research Effort:

This section reviews our progress on this project. As reviewed below, progress included (i) substantial improvement of techniques to record LC neurons, and to make local microinjections into the LC, in behaving monkeys, (ii) analyses of LC responsiveness to target cues during performance of a visual discrimination/vigilance task, (iii) analyses of changes in tonic LC activity with changes in attentiveness during task performance, (iv) characterization of LC activity and behavioral performance during acquisition (learning) a new cue-contingency during reversal training, (v) analysis of effects of local pharmacological activation of LC neurons on vigilance performance and attentiveness, (vi) collaboration with Dr. J. Cohen and David Servan-Schreiber of Carnegie Mellon University and U.

Pittsburgh to perform neural network analyses of LC function, and (vi) improvement in techniques to retrogradely label afferents to monkey LC.

1. Improvement of techniques to record and localize LC neurons in behaving monkeys. We have developed methods for using very small diameter (10 μm) microwires for unit recordings. This greatly improves the signal-to-noise and general quality of unit recordings from the LC or other brain regions compared to larger wires we had been using (25 μm diameter). This smaller size wire also allows multiple penetrations through the same small brain region with minimal damage. In addition, we have improved the cannula and microadvancer design so that we are considerably more accurate at placing electrodes in desired targets. Finally, we have developed novel microinjection techniques which allow remotely controlled microinjections of minute volumes (<100 nl) into the LC of the monkey during task performance, without disturbing the animal.

2. Analyses of LC responsiveness to target cues during performance of a vigilance task. Our studies of LC neurons during the vigilance task support our previous observations that these cells are selectively activated by target cues in the task (*). Quantitative analysis revealed that LC neurons were phasically activated by target cues at a surprisingly short latency (mean latency of ~100 msec). These latencies were significantly shorter than the latencies of behavioral responses to target cues, which were ~250-300 msec on average. Moreover, these latencies are among the shortest observed for brain neurons in response to discriminative stimuli, indicating that the LC response is in the early stages of brain circuits that process attention-related signals. In contrast, non-target stimuli elicited no significant activation of these same LC neurons in 100 trials. Similarly, no other LC neuron exhibited any apparent excitation following non-target stimuli when averaged over 100 trials. Weak excitatory responses to non-target stimuli were revealed in some neurons only when averaged over a very large number of trials (> 1000).

In addition, inspection of LC responses over time during the task revealed that response amplitudes to target cues varied periodically, in accordance with the animal's level of behavioral performance. During periods of poor behavioral performance LC responses to target cues were smaller in amplitude than during epochs of excellent behavioral performance; most cells failed to respond at all during periods of poor performance. Neurons which were recorded during substantial epochs of both excellent and poor performance (> 30 min each) were subjected to quantitative analysis which revealed that this difference between LC response magnitudes during poor vs. excellent behavioral performance was significant ($p<0.004$; $n = 6$ cells). It is noteworthy that LC responses to target cues were reduced during periods of poor performance even though these cues always yielded correct behavioral responses (hits).

The relationship between LC responses and behavioral responses were also analyzed in a trial-by-trial manner. Specifically, the latency of the first spike in an LC neuron following each target cue was compared to the latency of the corresponding behavioral response (lever release). This comparison was restricted to periods of excellent behavioral performance as LC responses to target cues only occurred during such times (as described above). This analysis revealed a highly significant positive correlation between LC response and behavioral response ($r = 0.30$; $p < 0.0001$; $n = 197$ trials for 5 cells), indicating that shorter latency LC responses were associated with shorter behavioral response latencies.

We also tested whether an LC response to a target cue altered the behavioral response to the following sensory cue. For this, we analyzed the rare occasions when the semi-randomly presented target cues occurred in pairs, and compared the latency of the lever releases for the first and second cues in each pair. All of these target cue pairs were preceded by a non-target cue in the stimulus series. Examination of the corresponding PSTHs confirmed that LC neurons were activated by each of the cues in these pairs; moreover, the magnitude of response for the first target cue was significantly greater than that for the second target cue of the pair ($p<0.05$). For the 7 sessions with 33 target-cue pairs examined, the lever response latency for the second target cue (260 + 7 msec) was significantly shorter than for the first cue (283 + 8 msec; $p<0.01$, paired two-tailed t-test). This indicates that behavioral responses to target cues that are preceded by a cue-evoked LC response were faster than behavioral responses to the same cues preceded by non-target stimuli which did not evoke LC responses. We also examined bar release latencies for target cues that were preceded by a non-target cue which either generated no response (correct skip) or an incorrect bar release (false alarm). This

analysis revealed that there was no consistent difference between bar release latencies for target stimuli depending upon whether the preceding trial elicited a bar release (8 sessions analyzed with 63 false alarm-target pairs and 1089 skip-target pairs). Thus, the shorter bar release latency for the second cue in the target cue pairs was not simply due to the preceding behavioral response.

LC neuronal responses to non-target stimuli that elicited lever releases (i.e., false alarms) were also analyzed. During periods of poor performance with frequent false alarms, LC neurons were not activated by non-target stimuli that evoked false alarm behavioral responses. Thus, none of the 17 cells analyzed exhibited a response to non-target stimuli that elicited either a behavioral response (false alarm) or no behavioral response (rejection; as described above). As noted above, the response of LC neurons to target stimuli that elicited lever releases (hits) was also markedly attenuated during periods of poor performance. Thus, LC neurons were relatively unresponsive overall during periods of poor behavioral performance.

In addition to these brief fluctuations in behavior, behavioral performance degraded with prolonged task activity, yielding a vigilance decrement typical of such tasks (Davies and Parasuraman, 1982; Parasuraman, 1984; Warm and Jerison, 1984). Comparison of performance in 30 min epochs at least 60 min apart during continuous task behavior revealed several changes in task performance in the later epochs compared to the early epochs: (i) the frequency of foveating the fix spot decreased substantially, (ii) the rate of false alarm responding increased significantly, and (iii) the latency of bar release for hits increased significantly, and became more variable. Responses of LC neurons also changed significantly between these same time epochs. LC responses to target cues in the later epochs of task performance were significantly smaller than for the same stimuli during the early epochs of task performance ($p<.01$). Thus, LC responses to target cues decreased significantly in parallel with behavioral performance during a time-dependent vigilance decrement.

Continuing experiments recording LC neurons during the vigilance task indicate that the activation of LC neurons by target cues is specifically related to the behavioral significance of the cue: (1) During a period of continuously excellent performance associated with high attentiveness LC responses to CS+ cues were large, whereas such responses virtually disappeared during poor performance, even for correct 'hit' trials. (2) LC responses also varied with target cue frequency, being smaller when target stimuli were presented at a probability of 0.5 compared to a probability of 0.1. (3) When targets were presented in pairs, the first (unexpected) target produced a significantly larger response than a second (expected) target. (4) In another experiment in which the target was cued by a preceding small visual stimulus, elevated LC activity preceded target presentation but no excitatory response followed for the target cue. (5) Infrequent (unexpected) non-target cues produced no response, similar to frequent non-target stimuli.

Our observations indicate that the response to target stimuli is not due to the frequency of stimulus presentation, to prediction of reward, or to motor performance, but rather depends on the urgency of the stimulus, i.e., on the perceived behavioral significance of the stimulus. This is consistent with the hypothesis that LC responses facilitate behavioral responses to CS+ and subsequent stimuli.

3. Analyses of changes in tonic LC activity with changes in attentiveness during vigilance performance. During drowsiness LC activity is very low (< 0.5 spikes/sec) and there is typically no task performance. We observed that during continuous alertness and task performance the frequencies of both LC discharge and successful foveation fluctuated over short (10-30 sec) and long time intervals (10-30 min). The long-term changes in LC discharge were consistently inversely correlated with task behavior, such that slightly elevated LC activity (by 0.5 to 1 spike/sec) was accompanied by decreased foveation frequency and poorer task performance. Correlation analyses revealed that this relationship was highly significant (of the cells analyzed to date, typically $r = -0.5$, $p < 0.001$). In addition, even short-term increases in LC tonic activity often corresponded to marked, similarly short-lasting reductions in foveation frequency. These results suggest that focused attentiveness varies with tonic LC discharge in an inverted U relationship. Very low LC activity is associated with drowsiness and inattentiveness, while high tonic LC discharge corresponds with labile attention and restlessness; optimal focusing of attention occurs with intermediate levels of tonic LC activity. Additional studies are underway to test whether fluctuations in tonic LC activity cause or reflect changes in attentiveness.

4. LC activity and behavioral performance during acquisition (learning) a new cue-contingency during reversal training. Impulse activity of LC neurons was recorded during the vigilance task and, to evaluate detailed aspects of lever responses, we recorded the signal from a strain gauge attached to the lever. The signal from another strain gauge attached to the head-mounted fixation post served to measure the animal's approach to the juice reward. As described above, LC neurons exhibited short-latency responses (about 100 ms onset) to target stimuli, while latencies of behavioral response were on the order of 300 ms. We found that both target and non-target stimuli elicited a slight lever release (below response threshold) until about 100 ms before the reaction time when the animal sharply released the lever for target trials or depressed it for non-target trials. This "decision point" was preceded by phasic LC activation for target stimuli by about 100 ms; no LC response occurred for non-target cues. In addition, during early trials after reversal of cue meaning animals frequently committed false alarms (lever releases to non-target cues). Lever release responses for both hits (correct responses to targets) and false alarms were followed by very similar approach signals from the fixation post strain gauge, suggesting that all lever releases were intentional and accompanied by anticipation of reward. However, after the first few trials LC cells were selectively activated only during hit trials; non-target cues did not elicit LC activation even though they frequently evoked false alarm behaviors. Also, LC activity did not vary with bar release outside of the task or with non-contingent delivery of juice reward. These findings indicate that (1) the LC may facilitate, but is not necessary for, behavioral responses. (2) LC responses do not reflect motor or pre-motor activity per se. (3) LC neurons rapidly alter their response in accordance with altered cue meaning, in advance of altered behavioral responses.

5. Conditioned responses of monkey locus coeruleus neurons anticipate acquisition of discriminative behavior in a vigilance task. Impulse activity was recorded extracellularly from noradrenergic neurons in the nucleus locus coeruleus of 2 cynomolgus monkeys performing a visual discrimination (vigilance) task. For juice reward, the subjects were required to rapidly release a lever in response to an improbable target stimulus (20% of trials; CS+) that was randomly intermixed with non-target (CS-) stimuli presented on a video display. As previously reported, all locus coeruleus neurons examined were phasically and selectively activated by target stimuli in this task. Other task events elicited no consistent response from these neurons (juice reward, lever release, fix spot stimuli, non-target stimuli). With reversal of the task contingency, locus coeruleus neurons ceased responding to the former target stimuli, and began responding instead to the new target (old non-target) stimuli. In addition, the latency of locus coeruleus response to target stimuli increased after reversal (by about 140 ms) in parallel with a similar increase in the latency of the behavioral response. These results indicate that the conditioned locus coeruleus responses reflect stimulus meaning and cognitive processing, and are not driven by physical sensory attributes. Notably, the reversal in locus coeruleus response to stimuli after task reversal occurred rapidly, hundreds of trials before reversal was expressed in behavioral responses. These findings indicate that conditioned responses of locus coeruleus neurons are plastic and easily altered by changes in stimulus meaning, and that the locus coeruleus may play an active role in learning the significance of behaviorally important stimuli.

6. Effects of local pharmacological activation of LC neurons on vigilance performance and attentiveness. Our studies reveal a close correlation between tonic LC activity and attentiveness, whereby tonically elevated LC activity corresponds to markedly decreased focused attention (measured by frequency of foveating a fix spot required to initiate each task trial; described above). To test whether this correlation reflects a causal relationship whereby elevated LC activity is responsible for decreased attentional focusing, we examined the effects of activating an LC nucleus by local pharmacological stimulation in a monkey performing the vigilance task. The cholinergic agonist pilocarpine (approx. 100-200 nl) was infused into the LC unilaterally during task performance. In the 2 experiments to date of this nature, pilocarpine injections led to a rapid and profound decrease in foveation frequency, as would be expected if elevated LC activity was responsible for decreased attentional focusing. We have now improved our microinfusion techniques to allow repeated tests with minimal damage, and will carry out similar experiments soon in the 2 monkeys we are currently studying.

7. Collaboration with Dr. J. Cohen of Carnegie Mellon to perform neural network analyses of LC function. We developed a simulation model of LC function and its influence on performance in the vigilance task. The model is described in detail in [*], and outlined briefly below. The model was a hybrid, with two basic components: a simple stimulus discrimination network that simulated performance in the behavioral task, and a detailed model of LC neuronal activity. The former was the simplest network model capable of performing the behavioral task, and was used to examine the influence of LC activity on performance. The model of LC was significantly more elaborate and biologically realistic, permitting a careful examination of the neural mechanisms that might be responsible for its different modes of functioning.

Stimulus discrimination network. This was composed of a small number of units, each of which represented cell assemblies supporting stimulus or response representations necessary for performing the task. Thus, there were two input units (for the target and distractor stimuli), two decision units, and one response unit. Only the target unit was connected to the response unit, based on the assumption that the animal was overtrained to respond to the target but not the distractor. Connections between units in different processing layers were excitatory (information flow) while those within a layer were inhibitory (competition), and activity of units was subject to small random variations (noise). Finally, the cortical action of NE was simulated as a change in the gain parameter of the activation function of processing units. Elsewhere, we have argued that this is consistent with a large body of data concerning the neurophysiological and behavioral effects of NE.

LC model. This was comprised of a population of 250 spiking neurons, each of which was a leaky integrate-and-fire cell that exhibited temporal dynamics similar to those obtained in detailed compartmental models. Each LC cell received input from the target decision unit, as well as noise which was responsible for a weak spontaneous firing rate of about 1 Hz [as observed in vivo. LC cells interacted with each other in two ways. First, lateral inhibition simulated the effect of local NE release . Second, we included electrotonic coupling among LC cells, which simulated such coupling found empirically (see below).

Simulation results. This model was able to capture the full set of neurophysiological and behavioral findings observed in the monkey experiments described above. The model explained the two modes of LC firing in terms of differences in the degree of electrotonic coupling within LC. High coupling caused stronger, more synchronized (phasic) activation of the LC in response to inputs due to the distribution of the high voltage in spiking cells to the remainder of the population across electrotonic links. At the same time, coupling reduced spontaneous firing by averaging uncorrelated noise among the population, resulting in an overall reduction in tonic activity. Thus, according to the model, changes in phasic and tonic firing properties are directly related to one another, and both are governed by the same mechanism: electrotonic coupling among LC units. The model also provided an explanation of the behavioral effects of LC. Reduced coupling produced high tonic activity, leading to higher unit responsivity (including a reduced response threshold), and thus a greater number of false alarms. Conversely, increased coupling led to a reduction of tonic activity and an increase in phasic activity. The reduction in tonic activity produced a reduction of unit responsivity (associated with an increase in response threshold), and thus a reduction in false alarms. However, this was compensated for by an increase in phasic activity which produced a temporary increase in responsivity shortly following targets, and therefore a quick response. Thus, an increase in electrotonic coupling was able to produce an increase in the accuracy of responding, without a cost in response time.

8. A new theory of LC function. The empirical and computational modeling studies reported above have led us to a new hypothesis of LC function. At the neurophysiological level, we propose that the LC governs the responsivity of its target neuronal assemblies to their afferent inputs. This can manifest overtly as the likelihood that a stimulus will elicit a behavioral response, or have internal consequences, such as on attentional selection. Thus, the influence that LC-evoked changes in responsivity have on performance will depend upon the type of task in which the organism is engaged (e.g., whether it demands behavioral and/or attentional responses). It will also depend on the magnitude and pattern of LC discharge. We hypothesize that the latter have broad functional consequences on behavior, by changing the attentional mode of the system. At the lowest levels of LC activity the system is unresponsive (note that, although this level of activity is of great interest for studies of sleep and arousal, it is not the object of the present application). At higher levels (intermediate tonic activity plus

stimulus-evoked phasic activity), the system becomes responsive to specific task-relevant stimuli. However, at the highest levels of tonic activity (with a concomitant reduction in phasic activity), responsivity is increased to the point that behavior (and attention) becomes indiscriminate and labile. We argue that each of these different modes has adaptive advantages, depending upon the current behavior of the animal and its relationship to the stimulus environment. Thus, the intermediate tonic mode (plus phasic activity) may serve to support cortically-focused behaviors optimized to specific stable environments, while the high tonic mode may provide the variability necessary to adapt to changing, or unpredictable environments. Our computational model provides explicit mechanisms for these effects, and their expression in specific behavioral tasks. These mechanisms include electrotonic coupling among LC neurons (regulating LC mode), and NE modulation of the gain of processing units targeted by the LC (regulating the responsivity of cortical units responsible for both behavioral and attentional processes). We have used these mechanisms to demonstrate that high coupling within the LC can produce intermediate tonic LC activity with robust phasic responses to significant stimuli, fostering focused, task-relevant, selective responding. In contrast, decreased coupling leads to high tonic LC discharge and reduced phasic activity, favoring less selective responsiveness to a broader class of stimuli, and thus more labile behavior. While behavioral lability may lead to impaired performance in tasks that require focused attention, according to our theory it should prove to be adaptive under other circumstances. Indeed, we believe that high tonic LC activity is critical to behavioral flexibility. For example, it may be necessary when imperative stimuli present themselves unexpectedly (e.g., a sudden threat), demanding a shift in the behavioral program, or when the current program is no longer effective in meeting its goals. In these circumstances, lability of responses may provide the variability necessary to engage in new behaviors. One of the goals of this project is to establish controlled circumstances under which this advantage of high tonic LC activity can be demonstrated experimentally.

9. Improvement in techniques to retrogradely label afferents to monkey LC. We have made injections of various retrograde tracers into the LC of several monkeys at the end of our recording experiments in these animals. We were surprised to find that many of the tracers used did not yield transport characteristic of that obtained in rat. Thus, attempts with wheat germ agglutinin-conjugated to horseradish peroxidase (WGA-HRP), Fluoro-Gold, or WGA-apoHRP-colloidal Gold did not yield transport of sufficient magnitude to allow study following large injections into the LC. However, our latest attempts employing the highly sensitive tracer cholera toxin b subunit (CTb) yielded robust retrograde labeling in each case following injections into the LC area of primates. We will use this tracer in the next animals following the physiology and behavioral experiments, and trace afferents to the primate LC.

Publications, Dec. 31, 1992-Dec. 30, 1995.
The following is a list of all publications during the reporting period.

Journal articles:

1. Van Bockstaele, E., Akaoka, H. and Aston-Jones, G., Brainstem afferents to the rostral (juxtafacial) nucleus paragigantocellularis: Integration of exteroceptive and interoceptive sensory inputs in the ventral tegmentum. Brain Res. 603: 1-8 (1993).

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17. Shiekhattar, R. and Aston-Jones, G., Activation of adenylate cyclase attenuates the hyperpolarization following single action potentials in brain noradrenergic neurons independently of protein kinase A. Neuroscience 62: 523-530 (1994).

18. Rajkowski, J., Kubiak, P. and Aston Jones, G., Activity of locus coeruleus neurons in monkey: Phasic and tonic changes correspond to altered vigilance. Brain Res. Bull. 35: 607-616 (1994).

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Book chapters:

1. Aston-Jones, G., Shiekhattar, R., Rajkowski, J., Kubiak, P. and Akaoka, H., Opiates influence noradrenergic locus coeruleus neurons by potent indirect as well as direct effects. In: The Neurobiology of Opiates, R. Hammer, ed., CRC Press, New York, pp. 175 - 202 (1993).

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Professional personnel.

Gary Aston-Jones, Ph.D., Professor & Direct, Div. Behav. Neurobiol. (PI)

Janusz Rajkowski, Ph.D., Research Assistant Professor

Piotr Kubiak, Ph.D., Postdoctoral Fellow

Jonathan Cohen, Ph.D., Assistant Professor, Carnegie Mellon University (no support from AFOSR)

Svetlana Ivanova, Ph.D., Research Associate

Interactions (meetings where we presented results of our studies supported by this project).

Chairman and speaker, "Catecholamines and Attention: New Basic, Clinical and Modeling Approaches", Workshop, Winter Conference on Brain Research, Whistler, British Columbia, January, 1993.

Speaker, Second International Symposium on the Neurobiology of the Locus Coeruleus, San Juan Islands, Washington, May 1993.

Speaker, Winter Conference on Brain Research, workshop speaker and panel speaker, Snowbird, Utah, January, 1994.

Speaker, International meeting, "Emotional Motor System", April, 1994, Schiermonnikoog, The Netherlands.

Co-chair and speaker, "PTSD: Current Biological and Behavioral Perspectives", symposium, American Psychiatric Association, Philadelphia, PA, May, 1994.

Invited Special Lecture, Association of Professional Sleep Societies, 8th Annual Meeting, Boston, June, 1994

Speaker (2 sessions), chairman of session "Neurobiology of the Stress Response in Noradrenergic Systems: Relevance to PTSD", Society of Biological Psychiatry, Philadelphia, May, 1994.

Co-chair and speaker, "Norepinephrine in the Prefrontal Cortex", Satellite Symposium to the Australian Psychological Society, Mudjimba, Queensland, September, 1994.

Speaker, McDonnell-Pew Workshop on Mechanisms of Arousal, Eugene, Oregon, October, 1994.

Speaker, Harvard Spring Symposium, "Neuromodulatory Systems and Behavior", Harvard University, May, 1995.

Co-chair and speaker, "Sleep and Attention: How Tight is the Connection?", Focus Group, 2nd International Congress of the World Federation of Sleep Research Societies, Nassau, September, 1995.

Speaker, Half-day session: "PTSD, Current Biological and Behavioral Perspectives", American Psychiatric Association conference, 1995 Institute on Psychiatric Services, Boston, October, 1995.

Speaker, "Catecholamines in the Prefrontal Cortex", session in symposium on association cortices, satellite to IBRO meeting, Japan, July, 1995.